

WHAT IS CLAIMED IS:

1. A method for analyzing data from hybridization of a sample to an array of oligonucleotide probes,
wherein the sample comprises a plurality of nucleotide sequences, each nucleotide sequence corresponding to a particular gene,
wherein some or all of the oligonucleotide probes are assigned to invertible subblocks such that each gene which hybridizes to an oligonucleotide probe assigned to a particular subblock does not hybridize to the oligonucleotide probes in the other subblocks, and
which method comprises a step of separately analyzing the data for the oligonucleotide probes in each subblock.
2. A method according to claim 1, wherein the array comprises a plurality (N_0) of oligonucleotide probes having a particular sequence length n so that all nucleic acid sequences having the particular sequence length are present on the array.
3. A method according to claim 2 wherein the particular sequence length n is from about 6 to about 20.
4. A method according to claim 3 wherein the particular sequence length n is from about 9 to about 16.
5. A method according to claim 4 wherein the particular sequence length n is from about 10 to about 12.
6. A method according to claim 4 wherein the particular sequence length n is from about 12 to about 15.

7. A method according to claim 1 wherein oligonucleotide probes are assigned to subblocks according to a method which comprises, for each subblock, steps of:

- (a) associating a gene g_a with a gene list for a subblock, wherein the gene g_a is not already associated with a gene list for a subblock; and
- (b) assigning an oligonucleotide probe o_x to the subblock, wherein the oligonucleotide probe o_x hybridizes to the gene g_a ,

wherein the steps are repeated for each subblock until each gene is associated with a gene list for a subblock.

8. A method according to claim 7, further comprising steps of:

- (c) for each probe o_x assigned to the subblock, associating genes g_b with the gene list for the subblock, wherein each gene g_b hybridizes to the probe o_x ; and
- (d) for each gene g_b associated with the gene list, assigning an oligonucleotide probe o_y to the subblock, wherein the oligonucleotide probe o_y hybridizes to the gene g_b .

9. A method according to claim 8 wherein the steps of:

- (c) associating genes g_b with the gene list for the subblock; and
- (d) assigning an oligonucleotide probe o_y for each gene g_b associated with the gene list

are iteratively repeated for each oligonucleotide probe o_y assigned in step (d).

10. A method according to claim 9 wherein the steps (c) - (d) are repeated for not more than 100 iterations.

11. A method according to claim 10 wherein the steps (c) - (d) are repeated for not more than 50 iterations.

12. A method according to claim 11 wherein the steps (c) - (d) are repeated for not more than 20 iterations.

13. A method according to claim 12 wherein the steps (c) - (d) are repeated for not more than 15 iterations.

14. A method according to claim 13 wherein the steps (c) - (d) are repeated for not more than ten iterations.

15. A method according to claim 14 wherein the steps (c) - (d) are repeated for not more than five iterations.

16. A method according to claim 15 wherein the steps (c) - (d) are repeated for not more than four iterations.

17. A method according to claim 16 wherein the steps (c) - (d) are repeated for not more than three iterations.

18. A method according to claim 17 wherein the steps (c) - (d) are repeated for not more than two iterations.

19. A method according to claim 9 wherein the steps (c) - (d) are iteratively repeated until, for each oligonucleotide probe o_x assigned to the particular subblock, all genes g_a that hybridize to the oligonucleotide probe o_x are associated with the gene list for the particular subblock.

20. A method according to claim 8 wherein:
- (i) each oligonucleotide probe assigned to a subblock has a degeneracy value indicating the number of different genes that hybridize to that oligonucleotide probe; and
 - (ii) the steps (c) - (d) are iteratively repeated until for each oligonucleotide probe o_x assigned to the particular subblock, all genes g_a that hybridize to the oligonucleotide probe o_x are associated with the gene list for the particular subblock.
21. A method according to claim 7 in which:
- (i) each oligonucleotide probe assigned to the subblock has a degeneracy value indicating the number of different genes that hybridize to that oligonucleotide probe, the degeneracy value being equal to or below a particular threshold T for each oligonucleotide probe assigned to the subblock; and
 - (ii) each gene g_a associated with the gene list for the subblock hybridizes to at least one oligonucleotide probe o_x having a degeneracy value less than the particular threshold T .
22. A method according to claim 1 wherein:
- (i) each oligonucleotide probe assigned to a subblock has a degeneracy value indicating the number of different genes that hybridize to that oligonucleotide probe, and
 - (ii) the degeneracy value is equal to or below a particular threshold T for each oligonucleotide probe assigned to the subblock.
23. A method according to claim 22 wherein the particular threshold T is no more than 100.

24. A method according to claim 23 wherein the particular threshold T is no more than 50.

25. A method according to claim 24 wherein the particular threshold T is no more than 20.

26. A method according to claim 25 wherein the particular threshold T is no more than ten.

27. A method according to claim 26 wherein the particular threshold T is no more than five.

28. A method according to claim 27 wherein the particular threshold T is no more than four.

29. A method according to claim 28 wherein the particular threshold T is no more than three.

30. A method according to claim 29 wherein the particular threshold T is no more than two.

31. A method according to claim 30 wherein the particular threshold T is one.

32. A method according to claim 1 in which expression levels are determined for each gene g_i that hybridizes to oligonucleotide probes assigned to a particular subblock by a method which comprises solving a system of linear equations for the hybridization of each gene g_i to each oligonucleotide probe o_j assigned to the particular subblock.

33. A method according to claim 32 wherein the system of linear equations is of the form

$$\vec{E} = (\hat{H}')^{-1} \cdot \vec{S}'$$

wherein:

- (a) each element E_i of the vector \vec{E} indicates abundance of a nucleotide sequence in the sample corresponding to a particular gene g_i ;
- (b) each element S_j of the vector \vec{S}' indicates a level of hybridization to a particular oligonucleotide probe o_j ; and
- (c) each element H_{ij} of the matrix \hat{H}' indicates hybridization affinity of the nucleotide sequence corresponding to said particular gene g_i for the particular oligonucleotide probe o_j .

34. A method according to claim 1 wherein each of the nucleotide sequences has a length ℓ_i equal to the length of the corresponding gene.

35. A method according to claim 1 wherein the length of each different nucleic acid is decreased before hybridization so that each different nucleic acid has a decreased length $L_i = \ell_i - \Delta L_i$, that is less than the length of the corresponding gene.

36. A method according to claim 35 wherein the length is decreased by enzymatic digestion.

37. A method according to claim 35 wherein the length of each different nucleic acid is decreased, on average, by a controlled amount $\langle \Delta L \rangle$.

38. A method according to claim 37 wherein the amount $\langle \Delta L \rangle$ is between about 50 and about 500 bases.

39. A method according to claim 38 wherein the amount $\langle \Delta L \rangle$ is between about 50-100 bases.

40. A method according to claim 38 wherein the amount $\langle \Delta L \rangle$ is between about 100-500 bases.

41. A method according to claim 35 wherein the length of each different nucleic acid is decreased by a method which comprises:

- (i) protecting each nucleic acid along a particular length; and
- (ii) removing the unprotected portion.

42. A method according to claim 35 wherein the average decreased length $\langle L \rangle$ is controlled.

43. A method according to claim 42 wherein the average decreased length $\langle L \rangle$ is less than or equal to about 500 bases.

44. A method according to claim 43 wherein the average decreased length $\langle L \rangle$ is less than or equal to about 100 bases.

45. A method according to claim 44 wherein the average decreased length $\langle L \rangle$ is about 50 bases.

46. A method according to claim 42 wherein the average decreased length $\langle L \rangle$ is between about 50 and 100 bases.

47. A method according to claim 42 wherein the average decreased length $\langle L \rangle$ is between about 100 and 500 bases.

48. A method for assigning all or some of a plurality of oligonucleotide probes to subblocks suitable for analyzing data from hybridization of a sample to an array of the oligonucleotide probes,

wherein the sample comprises a plurality of nucleotide sequences, each nucleotide sequence corresponding to a particular gene,

which method comprises steps of:

- (a) associating a gene g_a with a gene list for a subblock, wherein the gene g_a is not already associated with a gene list for a subblock; and
- (b) assigning an oligonucleotide probe o_x to the subblock, wherein the oligonucleotide probe o_x hybridizes to the gene g_a ,

wherein the steps are repeated for each subblock until each gene is associated with a gene list for a subblock.

49. A method according to claim 48 further comprising steps of:

- (c) for each probe o_x assigned to the subblock, associating genes g_b with the gene list for the subblock, wherein each gene g_b hybridizes to the probe o_x ; and
- (d) for each gene g_b associated with the gene list, assigning an oligonucleotide probe o_y to the subblock, wherein the oligonucleotide probe o_y hybridizes to the gene g_b .

50. A method according to claim 49 wherein the steps of:

- (c) associating genes g_b with the gene list for the subblock; and
- (d) assigning an oligonucleotide probe o_y for each gene g_b associated with the gene list

are iteratively repeated.

51. A method according to claim 50 wherein the step (c) - (d) are repeated for not more than 100 iterations.

52. A method according to claim 51 wherein the steps (c) - (d) are repeated for not more than 50 iterations.

53. A method according to claim 52 wherein the steps (c) - (d) are repeated for not more than 20 iterations.

54. A method according to claim 53 wherein the steps (c) - (d) are repeated for not more than ten iterations.

55. A method according to claim 54 wherein the steps (c) - (d) are repeated for not more than five iterations.

56. A method according to claim 55 wherein the steps (c) - (d) are repeated for not more than four iterations.

57. A method according to claim 56 wherein the steps (c) - (d) are repeated for not more than three iterations.

58. A method according to claim 57 wherein the steps (c) - (d) are repeated for not more than two iterations.

59. A method according to claim 50 wherein the steps (c) - (d) are iteratively repeated until, for each oligonucleotide probe o_x assigned to the particular subblock, all genes g_a that hybridize to the oligonucleotide probe o_x are associated with the gene list for the particular subblock.

60. A method according to claim 51 wherein:

- (i) each oligonucleotide probe assigned to a subblock has a degeneracy value indicating the number of different genes that hybridize to that oligonucleotide probe; and
- (ii) the steps (c) - (d) are iteratively repeated until, for each oligonucleotide probe o_x assigned to the particular subblock, all genes g_a that hybridize to the oligonucleotide probe o_x are associated with the gene list for the particular subblock.

61. A method according to claim 48 in which:

- (i) each oligonucleotide probe assigned to the subblock has a degeneracy value indicating the number of different genes that hybridize to that oligonucleotide probe, the degeneracy value being equal to or below a particular threshold T for each oligonucleotide probe assigned to the subblock; and
- (ii) each gene g_a associated with the gene list for the subblock hybridizes to at least one oligonucleotide probe o_x having a degeneracy value less than the particular threshold T .

62. A method according to claim 48 wherein:

- (i) each oligonucleotide probe assigned to a subblock has a degeneracy value indicating the number of different genes that hybridize to that oligonucleotide probe, and
- (ii) the degeneracy value is equal to or below a particular threshold T for each oligonucleotide probe assigned to the subblock.

63. A method according to claim 48, wherein the array comprises a plurality (N_0) of oligonucleotide probes having a particular sequence length n so that all nucleic acid sequences having the particular sequence length are present on the array.

64. A method for selecting a particular sequence length n for an array comprising a plurality (N_0) of oligonucleotide probes having the particular sequence length n , which method comprises:

- (a) identifying a sequence length n providing an average probe degeneracy $\langle d(n) \rangle$ suitable for analyzing nucleic acid expression using the array; and
- (b) selecting the identified sequence length n ,

wherein the average probe degeneracy $\langle d(n) \rangle$ indicates the number of different nucleic acids that hybridize, on average, to a particular oligonucleotide probe.

65. A method according to claim 64 wherein each of the different nucleic acids corresponds to a gene in a plurality (N_g) of different genes.

66. A method according to claim 65 wherein each of the nucleotide sequences has a length ℓ equal to the length of the corresponding gene.

67. A method according to claim 66 wherein the length of each different nucleic acid is decreased before hybridization so that each different nucleic acid has a decreased length $L_i = \ell_i - \Delta L_i$ that is less than the length of the corresponding gene.

68. A method according to claim 67 wherein the length is decreased by enzymatic digestion.

69. A method according to claim 67 wherein the length of each different nucleic acid is decreased, on average, by a controlled amount $\langle \Delta L \rangle$.

70. A method according to claim 69 wherein the amount $\langle \Delta L \rangle$ is between about 50 and about 500 bases.

71. A method according to claim 70 wherein the amount $\langle \Delta L \rangle$ is between about 50-100 bases.

72. A method according to claim 70 wherein the amount $\langle \Delta L \rangle$ is between about 100-500 bases.

73. A method according to claim 67 wherein the length of each different nucleic acid is decreased by a method which comprises:

- (i) protecting each nucleic acid along a particular length; and
- (ii) removing the unprotected portion.

74. A method according to claim 67 wherein the average decreased length $\langle L \rangle$ is controled.

75. A method according to claim 74 wherein the average decreased length $\langle L \rangle$ is less than or equal to about 500 bases.

76. A method according to claim 75 wherein the average decreased length $\langle L \rangle$ is less than or equal to about 100 bases.

77. A method according to claim 76 wherein the average decreased length $\langle L \rangle$ is about 50 bases.

78. A method according to claim 74 wherein the average decreased length $\langle L \rangle$ is between about 50 and 100 bases.

79. A method according to claim 74 wherein the average decreased length $\langle L \rangle$ is between about 100 and 500 bases.

80. A method according to claim 64 wherein the nucleic acids hybridize to the oligonucleotide probes with no more than a particular number (m) of base-pair mismatches.

81. A method according to claim 80 wherein the average probe degeneracy $\langle d(n) \rangle$ is provided by the relation

$$\langle d(n) \rangle = \frac{N_g}{N_0} (1 - n + \langle L \rangle) \times c$$

c is provided by the relation

$$c = \sum_{k=0}^m \binom{n}{k} 3^k, \text{ and}$$

$\langle L \rangle$ indicates the average length of the different nucleic acids.

82. A method according to claim 64 wherein the average probe degeneracy $\langle d(n) \rangle$ is provided by the relation

$$\langle d(n) \rangle = \frac{N_g}{N_0} (1 - n + \langle L \rangle) \times c$$

wherein $\langle L \rangle$ indicates the average length of the different nucleic acids, and c indicates the number of the different nucleic acids that hybridize, on average, to an oligonucleotide probe having the particular sequence length n .

83. A method according to claim 64 wherein the step (a) of identifying a sequence length n comprises:

- (i) comparing oligonucleotide sequences having a particular sequence length n with sequences of the different nucleic acids, so that

nucleic acids which hybridize to each oligonucleotide sequence are identified; and

- (ii) determining the average probe degeneracy $\langle d(n) \rangle$ from the number of different nucleic acids that hybridize to each oligonucleotide sequence.

84. A method according to claim 64 wherein the identified sequence length n provides an average probe degeneracy $\langle d(n) \rangle$ that is less than or equal to about five.

85. A method according to claim 64 wherein the identified sequence length n provides an average probe degeneracy $\langle d(n) \rangle$ that is less than or equal to about four.

86. A method according to claim 64 wherein the identified sequence length n provides an average probe degeneracy $\langle d(n) \rangle$ that is less than or equal to about three.

87. A method according to claim 64 wherein the identified sequence length n provides an average probe degeneracy $\langle d(n) \rangle$ that is less than or equal to about two.

88. A method according to claim 64 wherein the identified sequence length n provides an average probe degeneracy $\langle d(n) \rangle$ of about one.

89. A method according to claim 64 wherein the step (a) of identifying a sequence length n comprises:

- (i) assigning all or some of a plurality of oligonucleotide probes having a particular sequence length n to subblocks suitable for analyzing data from hybridization of a sample to an array of the oligonucleotide probes; and
- (ii) determining the average probe degeneracy $\langle d(n) \rangle$ from the oligonucleotide probes assigned to the subblocks.

90. A method according to claim 89 wherein the plurality of oligonucleotide probes is a plurality all nucleic acid sequences having the particular length n .

91. A method according to claim 89 wherein the oligonucleotide probes are assigned to subblocks according to a method which comprises steps of:

- (a) associating a gene g_a with a gene list for a subblock, wherein the gene g_a is not already associated with a gene list for a subblock; and
- (b) assigning an oligonucleotide probe o_x to the subblock, wherein the oligonucleotide probe o_x hybridizes to the gene g_a ,

wherein the steps are repeated for each subblock until each gene is associated with a gene list for a subblock.

92. A method according to claim 91 further comprising steps of:

- (c) for each probe o_x assigned to the subblock, associating genes g_b with the gene list for the subblock, wherein each gene g_b hybridizes to the probe o_x ; and
- (d) for each gene g_b associated with the gene list, assigning an oligonucleotide probe o_y to the subblock, wherein the oligonucleotide probe o_y hybridizes to the gene g_b .

93. A method according to claim 92 wherein the steps of:

- (c) associating genes g_b with the gene list for the subblock; and
- (d) assigning an oligonucleotide probe o_y for each gene g_b associated with the gene list

are iteratively repeated.

94. A method according to claim 93 wherein the step (c) - (d) are repeated for not more than 100 iterations.

95. A method according to claim 94 wherein the steps (c) - (d) are repeated for not more than 50 iterations.

96. A method according to claim 95 wherein the steps (c) - (d) are repeated for not more than 20 iterations.

97. A method according to claim 96 wherein the steps (c) - (d) are repeated for not more than ten iterations.

98. A method according to claim 97 wherein the steps (c) - (d) are repeated for not more than five iterations.

99. A method according to claim 98 wherein the steps (c) - (d) are repeated for not more than four iterations.

100. A method according to claim 99 wherein the steps (c) - (d) are repeated for not more than three iterations.

101. A method according to claim 101 wherein the steps (c) - (d) are repeated for not more than two iterations.

102. A method according to claim 93 wherein the steps (c) - (d) are iteratively repeated until, for each oligonucleotide probe o_x assigned to the particular subblock, all genes g_a that hybridize to the oligonucleotide probe o_x are associated with the gene list for the particular subblock.

103. A method according to claim 93 wherein

- (i) each oligonucleotide probe assigned to a subblock has a degeneracy value indicating the number of different genes that hybridize to that oligonucleotide probe; and
- (ii) the steps (c) - (d) are iteratively repeated until, for each oligonucleotide probe o_x assigned to the particular subblock, all genes g_a that hybridize to the oligonucleotide probe o_x are associated with the gene list for the particular subblock.

104. A method according to claim 91 in which:

- (i) each oligonucleotide probe assigned to the subblock has a degeneracy value indicating the number of different genes that hybridize to that oligonucleotide probe, the degeneracy value being equal to or below a particular threshold T for each oligonucleotide probe assigned to the subblock; and
- (ii) each gene g_a associated with the gene list for the subblock hybridizes to at least one oligonucleotide probe o_x having a degeneracy less than the particular threshold T .

105. A method according to claim 91 wherein:

- (i) each oligonucleotide probe assigned to a subblock has a degeneracy value indicating the number of different genes that hybridize to that oligonucleotide probe, and
- (ii) the degeneracy value is equal to or below a particular threshold T for each oligonucleotide probe assigned to the subblock.